

COMPOUNDS, PHARMACEUTICAL COMPOSITIONS AND THERAPEUTIC
METHODS OF PREVENTING AND TREATING DISEASES AND DISORDERS
ASSOCIATED WITH AMYLOID FIBRIL FORMATION

5 FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to compounds, pharmaceutical compositions and therapeutic methods of preventing and/or inhibiting fibril formation and more particularly to methods of preventing and/or treating amyloid - related diseases and disorders. The present invention further relates to methods of treating inflammations.

10 Proper protein folding is a crucial step required for normal functioning and turnover of proteins. However, various factors such as stress, specific genetic mutations and certain infections may induce a cascade of yet incompletely understood processes leading to conformational changes or misfolding of proteins and consequently to their abnormal accumulation as amyloid fibrils. Such conformational
15 changes often involve the conversion from an α -helix configuration to a β -pleated sheet structure. These structural rearrangements, followed by nucleation, polymerization, aggregation and fibril formation, play a central role in the pathogenesis of most neurodegenerative diseases, such as Alzheimer's, Huntington's, Parkinson's and prion diseases, as well as at least eight of the polyglutamine - related
20 disorders [Kaytor, M. D. and Warren, S. T. (1999) *J. Biol. Chem.* 274(53): 37507-10] and various amyloidosis syndromes (e.g., Multiple myeloma, Chronic inflammatory disease, Rheumatoid arthritis, Tuberculosis, Skin and lung abscesses, Cancer, Hodgkin's disease, Hemodialysis for CRF, Heredofamilial amyloidosis, Familial Mediterranean Fever and Familial amyloid polyneuropathy).

25 For example, Alzheimer's disease (AD) is characterized by the formation and progressive deposition of insoluble amyloid fibrils within the cerebral cortex. The key constituent of these amyloid deposits has been identified as a 39-43-amino acid long polypeptide, the β -amyloid peptide ($A\beta$). Once deposited as dense amyloid plaque cores, the peptide becomes highly resistant to further proteolysis and causes
30 dystrophy of the surrounding nerve cells [Knauer et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89(16): 7437-41; Nordstedt et al. (1994) *J. Biol. Chem.* 269(49): 30773-6]. However, it is still unclear whether the amyloid fibrils themselves or the soluble

oligomers of A β are the main neurotoxic species that contribute to neurodegeneration and dementia present in Alzheimer's disease or other amyloidosis – related disorders (De Felice FG, et al., 2004, FASEB J. 18: 1366-72).

Several studies aiming at identifying therapeutic approaches for preventing amyloid fibril formation have suggested the use of beta-sheet breaker such as N,N'-bis(3-hydroxyphenyl)pyridazine-3,6-diamine (RS-0406) to reverse amyloid beta-induced cytotoxicity (Nakagami Y, et al., 2002, Br. J. Pharmacol. 137: 676-82), the use of N-methylated derivatives to inhibit toxicity and protofibril formation in the amyloid-beta peptide beta(25-35) (Doig AJ, et al., 2002, Biochem. Soc. Trans. 30(4): 537-42), the mechanical unzipping of amyloid beta-fibrils (Kellermayer MS, et al., 2004, J. Biol. Chem. Epub ahead of print), the use of curcumin as an anti-inflammatory agent which suppresses amyloid accumulation (Yang F et al., 2004, J. Biol. Chem. Dec 7; Epub ahead of print), the use of a monoclonal antibody specific to the C-terminal 92-99 of beta(2)m (Motomiya Y, et al., 2005, Kidney Int. 67: 314-20) and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) to stabilize Transthyretin (TTR) tetramers (Miller SR, et al., 2004, Lab Invest. 84: 545-52).

Butyrylcholinesterase (BChE, EC 3.1.1.8) is the primary circulating cholinesterase, abundant in serum and present at synapses and neuromuscular junctions, where it binds the same structural unit as the synaptic variant of acetylcholinesterase (AChE), AChE-S, with which it shares C-terminal sequence homology. Like AChE, BChE is capable of hydrolyzing acetylcholine (ACh) at the end of each round of pre-synaptic secretion. However, while AChE has a narrow substrate specificity, BChE exhibits a wide specificity for both substrates and inhibitors.

Prior studies have shown that acetylcholinesterase (AChE) co-localizes with the A β peptides present in the brain of Alzheimer's patients [Inestrosa, N.C. et al. (1996a) Mol. Psychiatry 1(5): 359-61; Inestrosa, N. C. et al. (1996b) Neuron 16(4): 881-91;] and that amyloid β complexes including AChE are far more neurotoxic than A β aggregates alone [Alvarez et al. (1998) J. Neurosci. 18(9):3213-23]. Moreover, AChE, but not BChE, was found to promote aggregation of amyloid complexes [Inestrosa, 1996b (Supra)].

Despite advances in the field, there is still a great need to identify a suitable

therapeutic agent for preventing amyloid fibril formation.

SUMMARY OF THE INVENTION

While reducing the present invention to practice, the present inventors have
5 uncovered that BChE and more so BChE derived peptides are capable of preventing
and/or reversing amyloid fibril formation and thus can be used to prevent and/or treat
amyloidosis – related disorders and diseases. It was further found that BChE can
prevent or reduce inflammation.

According to one aspect of the present invention there is provided a method of
10 identifying a BChE derived peptide capable of preventing and/or reversing amyloid
fibril formation comprising contacting the BChE derived peptide with an amyloid
precursor protein and a β -sheet – responsive dye and measuring a fluorescence
intensity resulting from the β -sheet – responsive dye prior to and following the
contacting the BChE derived peptide with the amyloid precursor protein, wherein
15 delayed or reduced increase in the fluorescence intensity following the contacting the
BChE derived peptide with the amyloid precursor protein is indicative of an ability of
the peptide to prevent amyloid fibril formation. This is a high throughput method
which is readily automateable and which can be used to test, for example, within a
short time period each one of the peptides represented by SEQ ID NOs:8-20302, all
20 are BChE derived peptides.

According to further features in preferred embodiments of the invention
described below, the β -sheet – responsive dye is a benzothiazole dye.

According to still further features in the described preferred embodiments the
 β -sheet – responsive dye is Thioflavin T.

25 According to still further features in the described preferred embodiments the
Thioflavin T is provided at a concentration range of 0.5-1.5 μ M.

According to still further features in the described preferred embodiments the
Thioflavin T is provided at a concentration of about 1 μ M. As used herein throughout
the term “about” refers to ± 10 %.

30 According to still further features in the described preferred embodiments the
amyloid precursor protein is selected from the group consisting of Transthyretin,
Amyloid beta protein, Amyloid beta (1-40), Procalcitonin, IAPP (Amylin), amyloid